Femtosecond Fluorescence Dynamics of Tryptophan and 5-Fluorotryptophan in Monellin

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fluorophore via photoinduced electron transfer (PeT) [3]. By carefully tuning the redox potential of the BODIPY fluorophores, [4] we can have one redox state fluorescent, and the other quenched through PeT (e.g. reduced state off and oxidized state on). Furthermore we are able to tailor the BODIPY dyes to enhance lipid solubility [5] and target organelles. In this presentation I will discuss the general concepts behind the new probes and will focus on the reactivity and imaging opportunities in live cells arising from a novel α-tocopherol based fluorogenic probe that readily targets mitochondria [6]. I will also discuss the opportunities that a related probe based on a fluorogenic ubiquinone provides towards monitoring key metabolic processes within mitochondria.

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Femtosecond Fluorescence Dynamics of Tryptophan and 5-Fluorotryptophan in Monellin: Slow Water Relaxation Unmasked

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Time-resolved fluorescence decay profiles of single Trp/5FTrp monellins have been investigated using a ultraviolet upconversion spectrophotofluorometer with time resolution better than 150 fs together with a time correlated single photon counting apparatus on the 100 ps to 20 ns time scale. We analyzed the set of fluorescence decay profiles using the global analysis technique. Nanosecond fluorescence data evince multiexponential decay for either probe, but terms for the 5F derivative are generally longer lived. In the window below 100 ps, the time constant for bulk water ''solvent relaxation'' remains near 1 ~ 2 ps, with associated amplitude positive or negative depending on the emission wavelength *(J.A.C.S., 128, 1214, 2006).* A sub-20ps exponential component is consistently found for both probes; most important, only in 5F-Trp monellin one will find **negative** amplitudes at longer wavelengths (>360 nm). The negative amplitude preceding an exponential ''risetime'' is the hallmark of an excited state reaction or relaxation (e.g., evidence of an excited population building on the red side since bluer chromophores lose energy and shift redward). Native Trp in this setting showed positive amplitudes due to ultrafast quenching. Hence, water relaxations are unmasked due to either 5FW being refractory to ultrafast (nearby group) quenching or to unusual segmental movement of 5FTrp residues.

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Mechanistic Insights into Reversible Photoactivation in Proteins of the GFP Family

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Light-controlled modification of the fluorescence emission properties of proteins of the GFP family is of crucial importance for many imaging applications including super-resolution microscopy. Here we have studied the reversibly photoswitchable fluorescent protein mIrisGFP using optical spectroscopy. By analyzing the pH dependence of isomerization and protonation equilibria and the isomerization kinetics, we have obtained insight into the coupling of the chromophore to the surrounding protein moiety and a better understanding of the photoswitching mechanism. Isomerization and protonation are connected, and a different acid-base environment of the chromophore's protonating group in its two isomeric forms, which can be inferred from the x-ray structures of IrisFP, is key to the photoswitching function. Amino acids near the chromophore, especially Glu212, rearrange upon isomerization, and Glu212 protonation modulates the chromophore pKa. In mIrisGFP, the cis chromophore protonates in two steps, with pKcis of 5.3 and 6, which is much lower than pKtrans (>10). Based on these results, we have put forward a mechanistic scheme that explains how the combination of isomeric and acid-base properties of the chromophore in its protein environment can produce negative and positive photoswitching modes.

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Epidermal Growth Factor Receptor Localization in Cell Membranes Investigated by Imaging FCS

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The epidermal growth factor receptor (EGFR) is a receptor tyrosine kinase that plays essential roles in cell growth and proliferation. Despite many years of research there are still open questions about its mechanism of function. We have addressed earlier the question of EGFR dimerization as well as phosphorylation in live cells. Here we address in particular its distribution and dynamics on the cell membrane. The simultaneous elucidation of membrane organization and dynamics requires measurements with good spatiotemporal resolution. We recently introduced Imaging Total Internal Reflection Fluorescence Correlation Spectroscopy (ITIR-FCS) with time resolution down to 0.3 ms and diffraction-limited resolution to study whole membrane areas with single molecule sensitivity. ITIR-FCS can test the FCS diffusion laws and determine whether diffusion is free, takes place within a meshwork or is hindered by traps. We demonstrate the feasibility of this approach with various structured artificial bilayers. Advancing to live cell measurements, we compare the diffusive behavior of DiI (C12 and C18), a membrane marker for the fluid phase, GPI-GFP, a putative microdomain marker, transferring receptor-mCherry, a receptor interacting with the cytoskeleton, and the epidermal growth factor receptor-mRFP (EGFR-mRFP). We monitor the dynamics of the different molecules under conditions of cholesterol depletion (mbCD), cytoskeleton depolymerization (Latrunculin A), and EGF stimulation. EGFR clearly shows changes upon stimulation, implying stronger domain localization, with little sensitivity to depolymerization of the cytoskeleton and moderate sensitivity to cholesterol depletion.

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From Single-Molecule Fluorescence to Quantiative Structural Models of Dynamic Proteins: Human Guanylate Binding Protein 1

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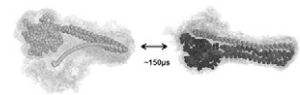
MFD (multiparameter fluorescence detection) is based on simultaneous recording of fluorescence lifetime, polarization, and color. hpFRET (high precision Forster resonance energy transfer) used in single-molecule experiment can show an existence of diverse conformations adopted by macromolecules.
fFCS (filtered Fluorescence Correlation Spectroscopy) is extended analysis method of time, polarization and color resolved FCS. The method uses MFD data to obtain information on the kinetics of transitions between fluorescence species on the multiple timescales (ns to ms).
The above techniques were applied to hGBP1 (the human guanylate binding protein 1) which is a GTP binding interferon-g induced protein important immuno defense. hGBP1 consists of three domains: a GTP-binding domain (gray), a middle domain (blue) and a helical domain (green). In solution it undergoes conformational transitions between a minor and a major state. The timescale of motion and structural models of the states were resolved by fFCS, MFD and hpFRET.



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Diffusion-Enhanced FRET between Membrane Proteins

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Förster resonance energy transfer (FRET) is often used to study interactions between membrane proteins. The use of long-lived (~10^{-3} sec) lanthanide donors allows for a delay between excitation and detection of emission, greatly enhancing the signal-to-noise ratio. However, this long excited state lifetime also allows donor- and acceptor-labeled membrane proteins to diffuse significant distances towards each other while the donor is excited, potentially enhancing energy transfer. Here we show that diffusion enhances lanthanide-based FRET (LRET) between membrane proteins, and that this phenomenon provides information concerning membrane protein organization. We constructed tetracycline-inducible cell lines expressing SNAP-tagged glycosylphosphatidylinositol (GPI)-anchored proteins (SNAP-GPI) and β_2 adrenergic receptors (SNAP-β_2AR), labeled these proteins with donor (Tb^{2+}) and acceptor fluorophores, and measured FRET while varying diffusion. The FRET signal (sensitized emission) between SNAP-GPI proteins decreased by 62 ± 3% ($P<0.001$; $n=4$; mean ± S.D.) when these proteins were immobilized with aldehyde fixation. Similarly, immobilization decreased FRET between